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(54) Title: METHOD OF REDUCING NEUROTOXIC INJURY WITH ZINC CHELATORS

(57) Abstract

The invention relates to the use of pharmaceutically acceptable zinc chelating compounds for the manufacture of medicaments for the treatment of neurotoxic injury.

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Method of reducing neurotoxic injury with zinc chelators

The invention relates to the use of zinc chelating compounds in the treatment of neurotoxic injury.

It has been discovered that zinc chelating compounds protect hippocampal neurons from the neurotoxic effects of Zn^{2+} released during neurotoxic events, such as ischemia as a result of stroke or cardiac arrest. Thus, the present invention comprises a method of treating neurotoxic injury in a patient suffering said injury, comprising administering to said patient a zinc chelating compound in an amount sufficient to treat said neurotoxic injury.

The administration of the composition comprising the zinc chelating compound is carried out by injection or infusion of the composition into the patients cerebrospinal fluid.

Chelatable Zn^{2+} is present in large quantities in presynaptic vesicles of central excitatory neurons (Danscher et al., 1985; Frederickson et al., 1983), and released with synaptic activity or membrane depolarization (Assaf and Chung, 1984; Howell et al., 1984; Charton et al., 1985). Although the precise role of released synaptic zinc is not known, it blocks NMDA receptor-mediated current (Westbrook and Mayer, 1987; Peters et al., 1987; Christine and Choi, 1990) and GABA receptor-mediated current (Westbrook and Mayer, 1987), as well as voltage-dependent calcium channels (Winegar and Lansman, 1990). In addition, exposure to excessive extracellular Zn^{2+} is neurotoxic to cortical neurons (Choi et al., 1988), possibly mediated by Zn^{2+} influx in part mediated by ionotropic glutamate receptors and voltage-gated calcium channels (Weiss et al., 1993; Koh and Choi, 1994). Recently, it has been shown that zinc translocates into degenerating hippocampal hilar neurons after transient ischemia (Tonder et al., 1990) or kainate-induced seizures (Frederickson et al., 1989).

Treating neurotoxic injury within the meaning of the present invention means reducing the extent of damage to central neurons surrounding a central neuron which has released Zn^{2+} due to its having been damaged by a

neurotoxic event. Neurotoxic events include acute neurological insults such as hypoxia/ischemia, such as occurs during stroke, cardiac arrest, hypoglycemia, epilepsy or trauma. Neurotoxic events may also be chronic neuronal damage caused by neurodegenerative disorders such as Huntington's disease, Alzheimer's disease, amyotropic lateral sclerosis, and the neurodegenerative effects of AIDS. Thus, the present invention also comprises a method of treating diseases, such as those described above, in which said neurotoxic injury occurs.

The zinc chelating compounds useful in accordance with the invention are not critical. Any conventional compound which is capable of chelating Zn^{2+} and which is pharmaceutically acceptable for injection into cerebral spinal fluid may be used in accordance with the invention.

While ethylenediaminetetraacetic acid (EDTA) is traditionally used as an organic chelating agent for Ca^{2+}, it has affinity for other divalent metal ions, and specifically binds Zn^{2+} with much higher affinity than Ca^{2+} (log stability constant at pH 7, 13.1 for Zn^{2+} versus 7.3 for Ca^{2+}). An equimolar combination for Ca^{2+} and EDTA will act as a chelating agent for Zn^{2+} without chelating local Ca^{2+} (Dansher et al., 1975). Thus, the preferred zinc chelating compound for use in accordance with the invention is disodium-calcium EDTA ("CaEDTA"), especially the form of CaEDTA known as edetate calcium disodium injection, USP (e.g., Calcium Disodium Versenate, 3M Pharmaceuticals, St. Paul, Minnesota).

Another preferred zinc chelating compound useful in accordance with the invention is 3-mercapto-D-valine (penicillamine), which is a chelating agent usually applied to the treatment of Wilson's disease where it removes excess copper.

Other zinc chelating compounds useful in accordance with the invention are:

bis(diethylthiocarbamoyl) disulfide (Disulfiram or Antabuse),
(ethylenedioxy)diethylenedinitrilotetraacetic acid (EGTA),
N,N,N',N'-tetrakis(2-pyridylmethyl)-ethylenediamine (TPEN),
N-(6-methoxy-8-quinolyl)-p-toluenesulfonamide (TSQ),
8-hydroxy quinoline (Oxyne),
8-hyroxy quinoline-5-sulphonic acid (Sulphoxine),
diethyl dithiocarbamate (DEDTC),

1,10-phenathroline,
dipicolinate,
N-acetyl cystein,
diphenylthiocarbazone (Dithizone),
1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2'-amino-5'-methylphenoxy)ethane-N,N,N',N'-tetraacetic acid (Fura-2), and
1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA).

The zinc chelating compound used in accordance with the present invention may be administered to the cerebrospinal fluid by any conventional means. The preferred method of administration is by injection or chronic pump infusion into the lateral ventricles of the patient's brain or into the lumbar sac of the patient.

The zinc chelating compound is preferably administered as a composition containing the zinc chelating compound and a pharmaceutically acceptable carrier compatible with the compound. In preparing such a composition, any conventional pharmaceutically acceptable carrier may be utilized. Typical carriers for administration by injection would be sterile aqueous/buffered solutions, preferably water for injecion or unbuffered or buffered physiological saline. The zinc chelating compound is preferably present in the carrier at a concentration of 1 mM - 300 mM, especially from 50 mM - 200 mM.

In carrying out the method of the invention, the zinc chelating compound is administered to adults daily in an amount from about 0.1 mg/kg to about 100 mg/kg daily, in single or divided doses, or continuously through chronic pump infusion. The preferred dosage will vary depending upon the indication for which the method of the invention is being used to treat. For treatment of patients who have suffered an acute neurotoxic insult, as a result of, e.g., stroke or cardiac arrest, the administration of a dosage from about 5 mg/kg to about 50 mg/kg daily, carried out for from 1 to 7 days, is preferred. Administration may be carried out by injection or by infusion utilizing a chronic infusion pump. For treatment of chronic neurotoxic conditions in patients, e.g., Alzheimer's disease, a dosage of from about1 mg/kg to about 10 mg/kg daily is preferred, carried out for months to years, preferably utilizing a chronic infusion pump.

Thus, the present invention also comprises a method of treating neurotoxic injury in a patient suffering said injury by administering to said

patient a composition comprising a zinc chelating compound and carrier, wherein both the compound and the carrier are pharmaceutically acceptable for injection. The prefered zinc chelating compounds are CaEDTA and 3-mercapto-D-valine, with CaEDTA being especially preferred. The zinc chelating compound is preferably administered in an amount from about 01. mg/kg to about 100 mg/kg daily, especially in an amount from about 5 mg/kg to about 50 mg/kg daily for the treatment of acute neurotoxic conditions and in an amount from about 1 mg/kg to about 10 mg/kg daily for chronic neurotoxic conditions. The preferred method of administration is the injection or infusion of the pharmaceutical composition comprising the zinc chelating compound and the carrier into the cerebrospinal fluid (e.g., the lateral ventricles or lumbar sac) of the patient. The preferred duration of treatment is from 1 to 7 days for acute neurotoxic conditions, and for months to years for chronic neurotoxic conditions.

The present invention preferably comprises a method of treating stroke in a patient suffering said stroke by administering to said patient a composition comprising a zinc chelating compound and a carrier, wherein both the compound and the carrier are pharmaceutically acceptable for injection. The preferred zinc chelating compounds are CaEDTA and 3-mercapto-D-valine, with CaEDTA being especially preferred. The zinc chelating compound is preferably administered in an amount from about 0.1 mg/kg to about 100 mg/kg daily, especially in an amount from about 5 mg/kg to about 50 mg/kg daily for the treatment of stroke. The preferred method of administration is the injection or infusion, especially injection, of the pharmaceutical composition comprising the zinc chelating compound and the carrier into the cerebrospinal fluid (e.g., the lateral ventricles or lumbar sac) of the patient. The preferred duration of treatment is from 1 to 7 days.

Experiments were conducted which determined that a zinc chelating compound, CaEDTA, exhibited neuroprotective effects in cell culture and animal models of brain hypoxic-ischemic injury, such as might occur consequent to cardiac arrest or stroke. Several observations were made:

1) In mouse cortical culture, CaEDTA selectively blocks Zn^{2+}- induced neuronal degeneration, but not the Ca^{2+}-overload neurotoxicity induced by glutamate agonists.

2) In a rat model of transient forebrain ischemia (bilateral carotid occlusion combined with hypotension), intraventricular injections of

CaEDTA, 30 minutes prior to the beginning of ischemia, reduced the ischemia-induced translocation of Zn^{2+} from presynaptic terminals throughout the brain to degenerating postsynaptic neurons. This CaEDTA treatment also markedly reduced the death of hippocampal hilar and CA1 pyramidal neurons.

Example 1

Use of disodium-calcium-EDTA (CaEDTA) in a rat model of transient global ischemia.

5 µl of 100 mM CaEDTA in saline, or saline alone, was injected into the lateral ventricles of Long Evans rats, 30 minutes prior to the inducement of 10 minutes of global ischemia. Ischemia was delivered by ligation of both common carotid arteries combined with hypotension after anesthesia (Smith et al., 1984).

Hippocampal sections from the treated and untreated rats were stained with the zinc-sensitive dye, TSQ (Frederickson et al., 1987). The examination of these sections showed that in the untreated rats zinc translocated from presynaptic inputs to degenerating hilar neuronal cell bodies between 3-24 hours after the ischemia. In the treated rats, CaEDTA blocked not only this zinc translocation, but also prevented subsequent hilar neuronal degeneration as assessed 3 days later.

TSQ staining also revealed the delayed appearance of zinc in CA1 pyramidal neuronal cell bodies of untreated rats 24-72 hours post ischemia. The CaEDTA treatment prior to the induced ischemia attenuated both the appearance of zinc in CA1 pyramidal neurons, and CA1 neuronal degeneration.

Control intraventricular injections of zinc EDTA, which is not a zinc chelating compound, did not alter the zinc translocation or hippocampal neuronal death following the ischemia in treated rats.

These results demonstrate that extracellular Zn^{2+} chelating compounds protect brain cells against neurotoxic injury which can be the result of ischemia or other neurodegenerative conditions associated with Zn^{2+} release.

Claims

1. The use of pharmaceutically acceptable zinc chelating compounds for the manufacture of medicaments for the treatment of neurotoxic injury.

2. The use of zinc chelating compounds according to claim 1, wherein the therapeutic indications include neurological insults such as ischemia as a result of stroke, cardiac arrest, hypoglycemia, epilepsy or trauma, neurodegenerative disorders such as Huntington's disease, Alzheimer's disease, amyotropic lateral sclerosis, and the neurodegenerative effects of AIDS.

3. The use of zinc chelating compounds according to claims 1 and 2, wherein the zinc chelating compounds are selected from the group consisting of
disodium-calcium-ethylenediaminetetraacetic acid,
3-mercapto-D-valine,
(ethylenedioxy)diethylenedinitrilotetraacetic acid (EGTA),
N,N,N',N'-tetrakis(2-pyridylmethyl)-ethylenediamine,
N-(6-methoxy-8-quinolyl)-p-toluenesulfonamide,
8-hydroxy quinoline,
8-hydroxy quinoline-5-sulphonic acid,
diethyl-dithiocarbamate,
bis(diethylthiocarbamoyl)-disulfide,
1,10-phenathroline,
dipicolinate,
N-acetyl cystein,
diphenylthiocarbazone,
1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2'-amino-5'-methylphenoxy)ethane-N,N,N',N'-tetraacetic acid and
1,2-bis(2-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid.

4. A medicament containing one or more zinc chelating compounds as defined in claim 3 and a pharmaceutically acceptable inert carrier for the treatment of neurotoxic injury which include neurological insults such as ischemia as a result of stroke, cardiac arrest, hypoglycemia, epilepsy or trauma, neurodegenerative disorders such as Huntington's disease, Alzheimer's disease, amyotropic lateral sclerosis, and the neurodegenerative effects of AIDS.

5. A method of treating neurotoxic injury in a patient suffering said injury by injecting or infusing into the cerebrospinal fluid of said patient a composition comprising a pharmaceutically acceptable zinc chelating compound as defined in claim 3 and a pharmaceutically acceptable carrier wherein said zinc chelating compound is present in said composition in an amount sufficient to treat said neurotoxic injury.

6. The method according to claim 5, wherein the zinc chelating compound is administered at a dosage in the range from about 0,1 mg/kg to about 100 mg/kg daily.

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